Recommendations for Clinical *CYP2C9* Genotyping Allele Selection: A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists

Victoria M. Pratt,*† Larisa H. Cavallari,*‡ Andria L. Del Tredici,*§ Houda Hachad,*¶ Yuan Ji,*‖ Ann M. Moyer,*,** Stuart A. Scott,*††‡‡ Michelle Whirl-Carrillo,*§§ and Karen E. Weck*¶¶

From the The Pharmacogenomics (PGx) Working Group of the Clinical Practice Committee*, Association for Molecular Pathology, Bethesda, Maryland; the Department of Medical and Molecular Genetics,† Indiana University School of Medicine, Indianapolis, Indiana; the Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics,‡ University of Florida, Gainesville, Florida; Millennium Health,§ LLC, San Diego, California; Translational Software,¶ Bellevue, Washington; the Department of Pathology and ARUP Laboratories,‖ University of Utah School of Medicine, Salt Lake City, Utah; the Department of Laboratory Medicine and Pathology,** Mayo Clinic, Rochester, Minnesota; the Department of Genetics and Genomic Sciences,†† Icahn School of Medicine at Mount Sinai, New York, New York; Sema4,‡‡ Stamford, Connecticut; the Department of Biomedical Data Science,§§ Stanford University, Stanford, California; and the Department of Pathology and Laboratory Medicine and Department of Genetics,¶¶ University of North Carolina, Chapel Hill, North Carolina

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Corresponding author:

Victoria M. Pratt, PhD

Indiana University School of Medicine, Department of Medical and Molecular Genetics, 975 West Walnut St. IB-350, Indianapolis IN 46202

Email: vpratt@iu.edu

ABSTRACT

The goals of the Association for Molecular Pathology Pharmacogenomics (PGx) Working Group of the Association for Molecular Pathology Clinical Practice Committee are to define the key attributes of PGx alleles recommended for clinical testing and a minimum set of variants that should be included in clinical PGx genotyping assays. This document provides recommendations for a minimum panel of variant alleles (Tier 1) and an extended panel of variant alleles (Tier 2) that will aid clinical laboratories when designing assays for *CYP2C9* testing. The Working Group considered functional impact of the variants, allele frequencies in different populations and ethnicities, the availability of reference materials, as well as other technical considerations for PGx testing when developing these recommendations. Our goal is to promote standardization of testing PGx genes/allele testing across clinical laboratories. These recommendations are not to be interpreted as restrictive but to provide a reference guide. The current document will focus on *CYP2C9* testing that can be applied to all *CYP2C9*-related medications. A separate recommendation on warfarin PGx testing is being developed to include recommendations on *CYP2C9* alleles and additional warfarin sensitivity–associated genes/alleles.

INTRODUCTION

The Association for Molecular Pathology (AMP) Pharmacogenomics (PGx) Working Group describes a minimum list of alleles to include in clinical cytochrome P450 2C9 (*CYP2C9*) genotyping panels. These recommendations are developed to guide clinical laboratory professionals who validate and offer clinical PGx assays, with the goal of promoting standardization of PGx testing across different laboratories. This series of AMP PGx Working Group documents should be implemented with other clinical guidelines such as those issued by the Clinical Pharmacogenetics Implementation Consortium (CPIC), which focus primarily on the interpretation of genotyping results and therapeutic recommendations for a specific drug(s).[1] The results of this study suggest variants for inclusion in clinical *CYP2C9* genotyping panels and defines the key attributes of those alleles that were chosen for recommendation in clinical PGx testing.

Clinical PGx testing assays across different laboratories differ with regard to both the star (*) allele haplotypes tested in each pharmacogene, as well as in the variants used to define those haplotypes.[2,3] A Genetic Testing Reference Material Program (GeT-RM) study[4] evaluated a number of PGx test panels across 28 genes in 137 genomic DNA samples, and found discrepant results between mostly attributable to assay design. Without exception, no two tests that examined any of the 28 PGx genes included in the study were designed to detect the same set of variants and/or haplotypes (alleles). This genotyping variability can result in discrepancies in haplotype and diplotype assignment, which may affect test interpretation, and ultimately patient care. For example, if a patient's genome harbors a heterozygous *CYP2C9* NM_000771.3:c.449G>A, p.Arg150His; rs7900194 variant, which defines the *8 (reduced function) allele common among African-Americans and Africans,[5,6] but the patient undergoes a test that does not include this variant, he or she may be assigned a genotype of *1/*1 (normal function) rather than *1/*8, impacting the predicted phenotype and clinical treatment strategy and potentially creating a health care disparity. Variability in the PGx alleles tested by different clinical laboratories has also led to discrepant results on proficiency testing (PT) surveys.[3]

The AMP PGx Working Group was formed to derive a minimum set of alleles/variants that should be included in clinical PGx genotyping test panels, and to define the key attributes of the selection of these alleles.

This group has previously published recommendations for variants that should be included in any clinical *CYP2C19* genotyping assay.[7] Through that effort, the committee developed a "two-tier" strategy and selection criteria for recommended PGx clinical testing. "Tier 1" recommended PGx variant alleles are those that i) have been well characterized and shown to significantly affect the function of the protein and/or gene leading to an alteration in a drug response phenotype, ii) have an appreciable minor allele frequency in a population/ethnicity group, and iii) have publicly available reference materials (RMs). Alleles/variants that currently meet at least one but not all three of the Tier 1 criteria are included as "Tier 2" variant alleles, which may be moved to Tier 1 if RMs or additional information becomes available. A description on the rationale for these clinical PGx testing recommendations and development of this two-tier classification strategy have been previously described in the *CYP2C19* recommendation document from this group.[7]

Gene: *CYP2C9*

The cytochrome P450 2C9 is a member of the CYP2C subfamily of the cytochrome P450 enzymes, and is one of the most abundant and important drug metabolizing enzymes. It has been estimated that ~15% of all CYP-related biotransformation is catalyzed by CYP2C9, including several widely prescribed medications with a narrow therapeutic index such as the anticoagulant warfarin and the anticonvulsant phenytoin.[8] Like other CYP enzymes, CYP2C9 catalyzes a variety of exogenous and endogenous compounds, many of which are also substrates for other phase I and/or phase II enzymes. Medications for which CYP2C9 is responsible for >25% of metabolic clearance have been summarized elsewhere.[9]

CYP2C9 is currently included in the Food and Drug Administration (FDA) Table of Pharmacogenetic Biomarkers in Drug Labeling for several FDA-approved drugs (https://www.fda.gov/Drugs/ScienceResearch/ucm572698.htm, last accessed 8/15/2018). The *CYP2C9* gene has nine exons and is located on chromosome 10q23.33 where several CYP2C subfamily members (*CYP2C18*, *CYP2C19*, *CYP2C9*, and *CYP2C8*) are clustered. Like other *CYP450* genes, *CYP2C9* is highly polymorphic and variant *CYP2C9* star (*) alleles are frequently included in clinical PGx testing assays. The two most well-characterized variant alleles are *CYP2C9*2* (NM_000771.3:c.430C>T, p.Arg144Cys, rs1799853) and *CYP2C9*3*

(NM_000771.3:c.1075A>C, p.Ile359Leu, rs1057910), both of which are associated with decreased enzyme activity and impaired drug metabolism phenotypes.[10] Among the 60 variant *CYP2C9* star (*) alleles listed on the PharmVar (The Pharmacogene Variation Consortium) website[11] (formerly "the Human Cytochrome P450 (CYP) Allele Nomenclature website"), at least 20 are reported to have *in vivo* and/or *in vitro* functional evidence of altered activity (https://www.pharmvar.org/gene/CYP2C9, last accessed 8/15/2018).

According to the Genetic Testing Registry, (https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=CYP2C9, last accessed 8/15/2018) and AMP Test Directory (https://www.amp.org/resources/test-directory/, last accessed 8/15/2018), the alleles included in *CYP2C9* genotyping tests offered by clinical laboratories in the United States range from a few targeted alleles to interrogation of the entire coding region, and the techniques include targeted genotyping, bidirectional Sanger sequencing, next-generation sequencing (NGS), whole genome sequencing (WGS), or whole exome sequencing (WES), with or without deletion/duplication analysis. Inconsistent clinical results across laboratories[12] may be attributed to the selection of tested *CYP2C9* alleles, targeted testing of populations with varying ethnic backgrounds, as well as technical performance of the testing platforms. Differences can also occur post-analytically, during PGx interpretation and/or reporting; however, addressing these issues is considered outside the scope of this series of AMP PGx recommendation documents.

Existing Guidelines

Clinical PGx guidelines are available from groups including CPIC (https://cpicpgx.org, accessed 8/15/2018), the Dutch Pharmacogenetics Working Group (DPWG) funded by the Royal Dutch Pharmacists Association[13] and the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) (http://cpnds.ubc.ca/, last accessed 8/15/2018). CPIC's (and others) goals are to address barriers to clinical implementation of pharmacogenetic tests by creating, curating, and posting freely available, peer-reviewed, evidence-based, updatable, and detailed gene/drug clinical practice guidelines (https://cpicpgx.org/). These guidelines, which were developed using extensive literature review and discussion among experts, are gene-drug pair oriented with an emphasis on interpretation of genotype and phenotype, and genotype-guided therapeutic recommendations. These documents have played a critical role in shaping the clinical implementation of PGx

tests and have facilitated development of clinical decision support tools for clinicians to better understand and more efficiently utilize PGx testing results. There are currently 10 guidelines available on CYP2C9-metabolized medications from the CPIC, DPWG, and CPNDS (PharmGKB,[14] https://www.pharmgkb.org/gene/PA126/guideline, last accessed 8/15/2018). Although some clinical PGx guidelines include summaries of known *CYP2C9* alleles, frequencies in various populations, and their functional and/or clinical relevance, they do not explicitly recommend specific variant alleles for clinical laboratories to include in *CYP2C9* genotyping panels. Moreover, while the FDA recognizes the role of *CYP2C9* genetic variability for a number of medications, very limited information is usually available in FDA product labels regarding the testing methods employed by drug manufacturers while conducting PGx studies. Despite the fact that many commercial platforms are available, clinical laboratories often develop their own laboratory tests for *CYP2C9* genotyping. Specific considerations from a diagnostic laboratory perspective such as allele selection, testing platforms, and availability of RMs have not been the focus of the consortia guidelines. However, consistency in clinical genotyping panels among clinical laboratories could further promote the utilization of these important clinical PGx practice guidelines.

The AMP PGx Working Group reviewed the variant *CYP2C9* star (*) alleles currently cataloged by PharmVar,[11] including allele function, multiethnic allele frequencies, the availability of RMs, and commercially available genotyping platforms (Table 1). Tier 1 recommended *CYP2C9* variant alleles were defined as those that have i) well-characterized alteration of CYP2C9 activity that has been shown to have an effect on drug response[15] and for which the functional variant is known, ii) appreciable minor allele frequency in a population (https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx, last accessed 8/15/2018), and iii) publicly available RMs (Table 2). Tier 2 *CYP2C9* variant alleles are defined as alleles that meet at least one but not all of the criteria for inclusion in Tier 1, and are considered optional for inclusion in expanded clinical genotyping panels. Some of the Tier 2 alleles may be recommended as Tier 1 in the future if RMs or additional information becomes available. Variants with unknown or uncertain function are not

recommended for inclusion in targeted clinical *CYP2C9* genotyping test panels, although it may be useful to include these in research panels to clarify functional and/or clinical outcomes.

Tier 1 *CYP2C9* Variant Alleles

CYP2C9 variant alleles recommended as Tier 1 by the AMP PGx Working Group include *CYP2C9 *2*, **3, *5, *6, *8,* and **11.* This recommendation was based on their well-established functional effects on CYP2C9 activity and drug response,[15] availability of RMs, and their appreciable allele frequencies in major ethnic groups (https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx, last accessed 8/15/2018). The *CYP2C9*2* and **3* alleles are the most common *CYP2C9* alleles interrogated by commercially available platforms (Table 1). Importantly, the inclusion of these two variants accounts for 98% to 100% of the currently defined variation in *CYP2C9* leading to decreased function in European, Middle Eastern, and Asian populations, but only ~25% of the currently defined *CYP2C9* variation in populations with African ancestry (Table 3). Thus, genotyping for only the *CYP2C9*2* and **3* variants will not detect the majority of *CYP2C9* genomic variation leading to decreased enzymatic activity in populations with African ancestry. The *CYP2C9*5, *6, *8*, and **11* alleles have a combined frequency of ~10% in populations with African ancestry and collectively are more common than the **2* and **3* alleles in these populations, accounting for ~75% of the currently defined *CYP2C9* variation in African and African-Americans. In contrast, these alleles have frequencies less than 0.4% in European and Asian populations (https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx, last accessed 8/15/2018), accounting for <3% of currently defined *CYP2C9* variation in those populations.

CYP2C9*2

The decreased function *CYP2C9*2* allele is characterized by the presence of a missense variant in exon 3 (NM_000771.3:c.430C>T, p.Arg144Cys, rs1799853) that causes a decrease in CYP2C9 enzymatic activity towards most of its substrates.[16–18] *CYP2C9*2* has an allele frequency ranging from 11% to 13% in European, Middle

Eastern, and South/Central Asian populations, but only ~2% in populations with African ancestry, and <1% in the East Asian population (https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx, last accessed 8/13/2018). This variant is also present in the *CYP2C9*35* allele that is defined by the additional presence of an arginine to leucine change at amino acid 125 (NM_000771.3:c.374G>T, p.Arg125Leu, rs72558189)[19] (https://www.pharmvar.org/gene/CYP2C9, accessed 8/15/2018). The functional effect of the *CYP2C9*35* defining variant is yet unknown.

*CYP2C9*3*

The decreased function *CYP2C9*3* allele has a missense variant in exon 7 (NM_000771.3:c.1075A>C, p.Ile359Leu, rs1057910) that causes significant reduction in enzymatic activity.[16,20] The magnitude of enzyme impairment caused by *CYP2C9*3* is more pronounced than that of *CYP2C9*2* for most important drug substrates.[21] Its frequency ranges from 7% to 10% in populations of European, Middle Eastern, and South/Central Asian ancestry, but is much lower in populations of African (~1%) and East Asian (~3%) ancestry (https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx, last accessed 8/13/2018). The *CYP2C9*3* defining variant (NM_000771.3:c.1075A>C, p.Ile359Leu, rs1057910) is also present in *CYP2C9*18*, which additionally harbors a missense variant (NM_000771.3:c.1190A>C, p.Asp397Ala, rs72558193). Very limited *in vitro* data are available on the functional effects of *CYP2C9*18*, which was initially identified in Indians.[22,23]

*CYP2C9*5*

The *CYP2C9*5* allele, which has been found almost exclusively in subjects of African descent, is characterized by a missense variant in exon 7 (NM_000771.3:c.1080C>G, p.Asp360Glu,rs28371686) and is associated with reduced enzymatic activity.[23]

*CYP2C9*6*

The *CYP2C9*6* allele is defined by a single nucleotide deletion in exon 5 that causes a frameshift (NM_000771.3:c.818delA, p.Lys273Argfs, rs9332131).[24] Although this allele has a lower frequency than the other decreased activity alleles among African-Americans and Africans (Table 3), its null activity and association with central nervous system phenytoin toxicity[24] and reduced warfarin dose requirements[25] makes it an important allele to interrogate when clinically genotyping *CYP2C9*.

*CYP2C9*8*

The *CYP2C9*8* allele is defined by a missense variant in exon 3 (NM_000771.3:c.449G>A, p.Arg150His, rs7900194) and is the most frequent decreased function allele among African-Americans and Africans (Table 3). The c.449G>A variant is very rare in most other populations (http://gnomad.broadinstitute.org/dbsnp/rs7900194, last accessed 8/15/2018). Although this allele results in decreased enzymatic function towards warfarin and phenytoin, it has been reported to exhibit substrate-specificity. For example, an *in vitro* study reported that it may confer increased enzymatic activity towards tolbutamide.[26] However, two promoter variants, NM_000771.3:c.-1766T>C (rs9332094) and NM_000771.3:c.-1188T>C (rs4918758), which are in strong linkage disequilibrium with the defining *CYP2C9*8* variant (rs7900194) and have been associated with decreased *CYP2C9* expression, may also contribute to the effects of the **8* allele.[27] Moreover, given the high homology in DNA sequence at the NM_000771.3:c.449G>A locus among CYP2C genes, genotyping for the NM_000771.3:c.-1766T>C polymorphism has been proposed as an alternative means of identifying *CYP2C9*8*.[28] Substrate specificity may limit the classification of this allele as a decreased function allele when assigning a likely phenotype if used to inform medications other than phenytoin or warfarin (eg, sulfonylureas).[6,29,30] Both *in vivo* and *in vitro* studies have shown that decreased warfarin clearance is associated with *CYP2C9*8*.[31] Although its functional impact is less well-characterized than the **2* and **3* alleles, the **8* allele was chosen as Tier 1 allele due to its prevalence in populations of African descent, among whom warfarin has been widely used. Due to concerns of substrate specificity for the *CYP2C9*8* allele, clinicians

should be cautious when interpreting genotypic results and taking clinical action for any substrate other than those that have been extensively characterized. Also of note, NM_000771.3:c. 449G>A/C/T is a tetraallelic variant also reported as NM_000771.3:c.449G>T, p.Arg150Leu, rs7900194 (*CYP2C9*27*) which should be taken into consideration when designing new assays or interpreting analytic results.

*CYP2C9*11*

The *CYP2C9*11* allele is defined by a missense variant (NM_000771.3:c.1003C>T, p.Arg335Trp, rs28371685) in exon 7. This allele has been reported across all ethnicities and is also prevalent among African-Americans and Africans (Table 3). Consistent with other Tier 1 recommended *CYP2C9* alleles, *CYP2C9*11* has been associated with impaired warfarin metabolism and lower dose requirements.[32]

Tier 2 *CYP2C9* Variant Alleles

The following *CYP2C9* alleles are recommended for inclusion in Tier 2: *CYP2C9*12, *13*, and **15* (Table 4). The *CYP2C9*12* allele is defined by a missense variant in exon 9 (NM_000771.3:c.1465C>T, p.Pro489Ser, rs9332239); *CYP2C9*13* is defined by a missense variant in exon 2 (NM_000771.3:c.269T>C, p.Leu90Pro, rs72558187); and *CYP2C9*15* is defined by a nonsense variant in exon 4 (NM_000771.3:c.485C>A, p.Ser162*, rs72558190). These alleles have been shown to have either decreased function or no function (https://www.pharmvar.org/gene/CYP2C9, last accessed 8/15/2018) towards major CYP2C9 substrates and therefore may be included in more comprehensive clinical genotyping panels. However, they were not included in the Tier 1 recommendations due to either very low multi-ethnic minor allele frequencies (<0.5%) and/or a lack of currently available RMs (**13* and **15*). As further information is available for these variants and RMs become available, they may be promoted to Tier 1 *CYP2C9* recommended alleles.

DISCUSSION

Professional organizations such as AMP devote resources and efforts to establishing recommendations for professional practice, as it is important for molecular diagnostic laboratories to have resources for developing and validating clinical diagnostic testing. AMP members are among the early adopters and users of PGx testing in clinical settings and have accumulated substantial knowledge and expertise that is useful for laboratories beginning to implement these tests. This document offers a two-tier categorization of recommended *CYP2C9* alleles for inclusion in clinical *CYP2C9* genotyping assays.

The AMP PGx Working Group has proposed a recommended minimum set of alleles and their defining variants (Tier 1) that should be included in clinical *CYP2C9* genotyping tests based on allele function, population frequency, and the availability of RMs. In addition, the Working Group has defined selected *CYP2C9* alleles that do not currently meet one or more of the criteria for inclusion in Tier 1 and are thus considered optional for clinical testing (Tier 2). These recommendations are intended to facilitate standardization of testing by laboratories and to improve genotyping concordance across laboratories.

The Tier 1 alleles recommended for clinical testing were selected based on their reported clinical relevance for CYP2C9-associated medications, their frequency, and the availability of RMs. Tier 1 alleles include: *CYP2C9*2*, **3*, **5*, **6*, **8* and **11*. *CYP2C9*2* and **3* are the most common alleles in Caucasians and Asians (Table 3) and have been extensively investigated among CYP2C9-metabolized medications with narrow therapeutic ranges such as warfarin and phenytoin. Similar data exist for the **5, *6, *8*, and *11 alleles, which occur predominately in populations of African descent.

There are currently two published CPIC practice guidelines that involve *CYP2C9*, and both also incorporate additional pharmacogene(s): warfarin (*CYP2C9, VKORC1, CYP4F2*, and rs12777823)[15] and phenytoin (*CYP2C9* and *HLA-B*).[33] A CPIC practice guideline for *CYP2C9* and celecoxib is in progress (personal communication, K. Caudle). A second PGx expert group led by the Royal Dutch Association for the Advancement of Pharmacy, the Dutch pharmacogenetics work group (DPWG), offers additional guidance for use of *CYP2C9* genotyping for prescribing acenocoumarol (not FDA-approved), glibenclamide, gliclazide, glimepiride, phenoprocoumon (not FDA approved), and tolbutamide.[34] Moreover, FDA approved labeling information also includes dosing

recommendations based on *CYP2C9* genotype for the following medications: celecoxib (https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/020998s048lbl.pdf, last accessed 8/15/2018), flibanserin (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/022526lbl.pdf, last accessed 8/15/2018), flurbiprofen (https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/018766s020lbl.pdf, last accessed 8/15/2018), lesinurad (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/207988lbl.pdf, last accessed 8/15/2018), phenytoin (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/084349s081s082s084lbl.pdf, last accessed 8/15/2018), siponimod (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/209884s000lbl.pdf, last accessed 4/2/2019) and warfarin (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/009218s118lbl.pdf, last accessed 8/15/2018). As such, *CYP2C9* testing has the potential to guide clinicians when considering the use of at least 12 different medications, some of which are among the top 200 most prescribed medications in the US (http://clincalc.com/DrugStats/, last accessed 8/15/2018).

Although this document provides allele recommendations for all clinical *CYP2C9* genotyping indications, *CYP2C9* testing is closely associated with warfarin dosing. The CYP2C9 enzyme metabolizes the more potent *S*-warfarin enantiomer, and the *CYP2C9 *2, *3, *5, *6, *8*, and **11* alleles are associated with reduced *S*-warfarin clearance.[23,31,32,35–37] Data consistently demonstrate reduced warfarin dose requirements in individuals that carry these variant alleles, and a recent clinical trial showed that warfarin dosing guided by *CYP2C9*, *VKORC1*, and *CYP4F2* genotypes reduced the composite endpoint of venous thromboembolism, major bleeding, supratherapeutic anticoagulation, and death compared to a non-genotype guided approach.[38] The FDA-approved warfarin labeling includes dosing recommendations based on *CYP2C9* and *VKORC1* genotype.[15] However, the prescribing information does not include the alleles that have been shown to be important for warfarin response in patients of African descent. This is especially relevant for ethnically diverse and admixed populations. African alleles may be present in individuals that may not consider themselves of African descent; thus, determination of African ethnicity may not be required to test for these alleles. When these alleles are

present in any patient regardless of known African descent, they would be associated with reduced metabolism and could be used for therapeutic decisions. Failing to account for the *CYP2C9*5, *6, *8*, and **11* alleles in warfarin PGx dosing algorithms may lead to increased risk for over-dosing in African Americans.[15,39,40] In addition, current CPIC guidelines for warfarin dosing underscore the importance of accounting for African alleles and provide separate recommendations for patients of African versus non-African ancestry.[15] As such, we recommend that laboratories include all Tier 1 alleles, including the major African alleles, in clinical *CYP2C9* testing.

The CYP2C9 enzyme is also involved in the metabolism of phenytoin. Patients with a reduced or no function allele (eg, **2, *3, *5, *6, *8, *11)* are more likely to have impaired metabolism of phenytoin and require lower doses of the drug to prevent neurologic toxicity.[30,33,41] CPIC guidelines recommend consideration of lower phenytoin doses in patients who carry reduced function *CYP2C9* alleles.[33]

The Tier 2 recommended *CYP2C9* alleles are additional variant alleles that laboratories may choose to include in expanded clinical genotyping assays. The three Tier 2 *CYP2C9* alleles have been shown to have reduced or no function. However, *CYP2C9*13* and **15* lack available RMs, and all three alleles have very low minor allele frequencies (<0.3%) in major ethnic groups as described above. In particular, the *CYP2C9*15* no function allele has been found in East Asian (Exome Aggregation Consortium, http://exac.broadinstitute.org/, last accessed 8/9/2018) and South Asian populations[22,42] at very low frequencies (<0.01%), however, testing of this allele may more accurately assign phenotype for these ethnic groups.

The AMP PGx Working Group considered additional *CYP2C9* star (*) alleles for possible inclusion in Tier 2, including *CYP2C9*4, *7, *9, *10, *14, *18, *25, *31*, and **35.* These were not included in Tier 2 at this time for the following reasons: *CYP2C9*4, *10,* and **31* are extremely rare and have no appreciable allele frequency in multiethnic populations. *CYP2C9*7, *10,* and **14* are of uncertain function currently, and therefore are not recommended for inclusion in clinical testing panels, although it may be useful to include these alleles in research studies designed to measure their phenotypic effect. *CYP2C9*9* is a normal function allele most frequent in African populations, with a minor allele frequency of up to 7.5% (Exome Aggregation Consortium,

http://exac.broadinstitute.org/, last accessed 8/9/2018); however, since this allele has no effect on enzymatic activity, it was not included in recommended clinical test panels as failure to detect it would have no clinical significance. *CYP2C9*18* is a recently described variant of unknown allele frequency that shares the **3* allele variants, with the addition of a NM_000771.3:c.1425A>T alteration resulting in a p.Asp397Ala amino acid change, and is likely a sub-allele of *CYP2C9*3*. Similarly, *CYP2C9*35* shares the *CYP2C9*2* variant as well as having NM_000771.3:c.374G>T (p.Arg125Leu).[19] More information is necessary regarding the functional significance and allele frequency of both *CYP2C9*18* and *CYP2C9*35* before they could be recommended by the Working group for inclusion in clinical genotyping panels. Although *CYP2C9*25* is a frameshift deletion of 10 nucleotides resulting in loss of function, currently there is no allele frequency information available and it is likely to be extremely rare. In addition, for many of these alleles (*CYP2C9*4, *7, *14, *25,* and **31*) there are currently no publicly available RMs.

The AMP PGx Working Group identified seven commercially available platforms for *CYP2C9* genotyping at the time of this publication. All of these platforms include **2* and **3*; however, only two include all recommended Tier 1 alleles, including the African alleles *5, *6, *8, and *11, as well as the Tier 2 alleles (note open, platforms such as OpenArray from ThermoFisherh (Waltham MA) may be customized to include all Tier 1 and Tier 2 alleles). The PGx Working Group is aware that the recommendations to include the alleles more prevalent among African and African-American populations may be difficult to implement with currently available genotyping platforms. However, the Working Group concluded that failure to include these alleles could lead to inaccurate CYP2C9 phenotype prediction among individuals with known or unknown African ancestry and may contribute to existing health care disparities in these populations. Implementation of this recommendation document is at the discretion of the laboratory. The Tier 1 alleles are currently included in the available proficiency testing (PT) programs [eg, College of American Pathologists (http://www.cap.org/web/home/lab/proficiency-testing?_adf.ctrl-state=8oixhrwfk_4&_afrLoop=116815371771304#, last accessed 8/15/2018), and the North American Specialized Coagulation Laboratory Association (https://www.nascola.com/AccessibleServices/Testing, last

accessed 8/15/2018)]. The majority of laboratories participating in these PT programs currently test for all of the Tier 1 alleles (CAP Biochemical Molecular Genetics Committee, PGX A, 2017 and PGXB, 2017 PT Surveys, College of American Pathologists, 2017). This indicates that many clinical laboratories are already testing the Tier 1 alleles and that these recommendations would be practical to implement and reinforce standardization between laboratories. Of note, FDA has recently approved a direct-to-consumer pharmacogenetics test which includes most of the CYP2C9 Tier 1 alleles (https://www.accessdata.fda.gov/cdrh_docs/pdf18/DEN180028.pdf, accessed 11/5/2018), but does not include CYP2C9 *8 and *11, which together are found in approximately 8% of individuals with African ancestry (https://www.pharmgkb.org/page/cyp2c9RefMaterials, accessed 11/5/2018).

This AMP document is limited to recommendations for clinical laboratory testing. It does not include, for example, mapping of genotypes to phenotype (metabolizer status), clinical interpretation of *CYP2C9* genotyping, or recommendations for changes to medication therapy based on *CYP2C9* genotype. Prediction of CYP2C9 metabolizer status based on genotype and recommendations for clinical actions based on CYP2C9 phenotype are covered by guidelines published by CPIC and other professional groups. PGx is a rapidly changing field, and we intend to update these recommendation documents as new data and/or RMs become available. The AMP PGx Working Group recognizes that there are additional alleles that are not listed in this document; there are over 60 *CYP2C9* alleles currently listed in the PharmVar database (https://www.pharmvar.org/gene/CYP2C9, last accessed 8/15/2018). Some of these may be updated to Tier 2 or Tier 1 recommended alleles in the future based on new data concerning functional impact, frequency, and availability of RMs.

In summary, this AMP document provides recommendations for a list of alleles that should be included in clinical *CYP2C9* genotyping tests. These recommendations are intended to facilitate *CYP2C9* genetic testing by clinical laboratories. In addition, these recommendations should help to standardize testing and genotyping concordance between laboratories.

Disclaimer

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References

1. Relling M: Clinical implementation of pharmacogenetics: CPIC guidelines. Clin Chem Lab Med 2015, 53:S75.

2. Moyer AM, Rohrer Vitek CR, Giri J, Caraballo PJ: Challenges in Ordering and Interpreting Pharmacogenomic Tests in Clinical Practice. Am J Med 2017, 130:1342–1344.

3. Wu AHB: Genotype and phenotype concordance for pharmacogenetic tests through proficiency survey testing. Arch Pathol Lab Med 2013, 137:1232–1236.

4. Pratt VM, Everts RE, Aggarwal P, Beyer BN, Broeckel U, Epstein-Baak R, Hujsak P, Kornreich R, Liao J, Lorier R, Scott SA, Smith CH, Toji LH, Turner A, Kalman L V.: Characterization of 137 Genomic DNA Reference Materials for 28 Pharmacogenetic Genes: A GeT-RM Collaborative Project. J Mol Diagnostics 2016, 18:109–123.

5. Scott SA, Jaremko M, Lubitz SA, Kornreich R, Halperin JL, Desnick RJ: CYP2C9*8 is prevalent among African-Americans: Implications for pharmacogenetic dosing. Pharmacogenomics 2009, 10:1243–1255.

6. Cavallari LH, Langaee TY, Momary KM, Shapiro NL, Nutescu EA, Coty WA, Viana MAG, Patel SR, Johnson JA: Genetic and clinical predictors of warfarin dose requirements in African Americans. Clin Pharmacol Ther 2010, 87:459–464.

7. Pratt VM, Del Tredici AL, Hachad H, Ji Y, Kalman L V., Scott SA, Weck KE: Recommendations for Clinical CYP2C19 Genotyping Allele Selection: A Report of the Association for Molecular Pathology. J. Mol. Diagnostics. 2018, pp. 269–276.

8. Isvoran A, Louet M, Vladoiu DL, Craciun D, Loriot MA, Villoutreix BO, Miteva MA: Pharmacogenomics of the cytochrome P450 2C family: impacts of amino acid variations on drug metabolism. Drug Discov. Today. 2017, pp. 366–376.

9. Daly AK, Rettie AE, Fowler DM, Miners JO: Pharmacogenomics of CYP2C9: Functional and clinical

considerations. J. Pers. Med. 2018, .

10. Van Booven D, Marsh S, McLeod H, Carrillo MW, Sangkuhl K, Klein TE, Altman RB: Cytochrome P450 2C9-CYP2C9. Pharmacogenet. Genomics. 2010, pp. 277–281.

11. Gaedigk A, Ingelman-Sundberg M, Miller NA, Leeder JS, Whirl-Carrillo M, Klein TE: The Pharmacogene Variation (PharmVar) Consortium: Incorporation of the Human Cytochrome P450 (CYP) Allele Nomenclature Database. Clin Pharmacol Ther 2018, 103:399–401.

12. Pratt VM, Zehnbauer B, Wilson JA, Baak R, Babic N, Bettinotti M, Buller A, Butz K, Campbell M, Civalier C, El-Badry A, Farkas DH, Lyon E, Mandal S, McKinney J, Muralidharan K, Noll L, Sander T, Shabbeer J, Smith C, Telatar M, Toji L, Vairavan A, Vance C, Weck KE, Wu AHB, Yeo KTJ, Zeller M, Kalman L: Characterization of 107 genomic DNA reference materials for CYP2D6, CYP2C19, CYP2C9, VKORC1, and UGT1A1: a GeT-RM and Association for Molecular Pathology collaborative project. J Mol Diagnostics 2010, 12:835–846.

13. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, Rongen GAPJM, van Schaik RHN, Schalekamp T, Touw DJ, van der Weide J, Wilffert B, Deneer VHM, Guchelaar H-J: Pharmacogenetics: From Bench to Byte— An Update of Guidelines. Clin Pharmacol Ther 2011, 89:662–673.

14. Whirl-Carrillo M, McDonagh EM, Hebert JM, Gong L, Sangkuhl K, Thorn CF, Altman RB, Klein TE: Pharmacogenomics Knowledge for Personalized Medicine. Clin Pharmacol Ther 2012, 92:414–417.

15. Johnson JA, Caudle KE, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, Lee MT, Gage BF, Kimmel SE, Perera MA, Anderson JL, Pirmohamed M, Klein TE, Limdi NA, Cavallari LH, Wadelius M: Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update. Clin Pharmacol Ther 2017, 102:397–404.

16. Rettie AE, Haining RL, Bajpai M, Levy RH: A common genetic basis for idiosyncratic toxicity of warfarin and phenytoin. Epilepsy Res 1999, 35:253–255.

17. Rettie AE, Wienkers LC, Gonzalez FJ, Trager WF KK: Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. Pharmacogenetics 1994, 4:39–42.

18. Crespi CL, Miller VP: The R144C change in the CYP2C9*2 allele alters interaction of the ctochrome P450 with NADPH:cytochrome P450 oxidoreductase. Pharmacogenetics 1997, 7:203–210.

19. Ciccacci C, Falconi M, Paolillo N, Oteri F, Forte V, Novelli G, Desideri A, Borgiani P: Characterization of a novel CYP2C9 gene mutation and structural bioinformatic protein analysis in a warfarin hypersensitive patient. Pharmacogenet Genomics 2011, 21:344–346.

20. Steward DJ, Haining RL, Henne KR, Davis G, Rushmore TH, Trager WF, Rettie AE: Genetic association between sensitivity to warfarin and expression of CYP2C9*3. Pharmacogenetics 1997, 7:361–367.

21. Hiratsuka M: Genetic Polymorphisms and in Vitro Functional Characterization of CYP2C8, CYP2C9, and CYP2C19 Allelic Variants. Biol Pharm Bull 2016, 39:1748–1759.

22. Zhao F, Loke C, Rankin SC, Guo JY, Lee HS, Wu TS, Tan T, Liu TC, Lu WL, Lim YT, Zhang Q, Goh BC, Lee SC: Novel CYP2C9 genetic variants in Asian subjects and their influence on maintenance warfarin dose. Clin Pharmacol Ther 2004, 76:210–219.

23. Niinuma Y, Saito T, Takahashi M, Tsukada C, Ito M, Hirasawa N, Hiratsuka M: Functional characterization of 32 CYP2C9 allelic variants. Pharmacogenomics J 2014, 14:107–114.

24. Kidd RS, Curry TB, Gallagher S, Edeki T, Blaisdell J, Goldstein JA: Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. Pharmacogenetics 2001, 11:803–808.

25. Quinn AL, Liko I LJ: Clinical effect of CYP2C9*5/*6 genotype on a patient's warfarin dose requirement. Pharmacogenomics 2017, 18:1051–1057.

26. Blaisdell J, Jorge-Nebert LF, Coulter S, Ferguson SS, Lee SJ, Chanas B, Xi T, Mohrenweiser H, Ghanayem B, Goldstein JA: Discovery of new potentially defective alleles of human CYP2C9. Pharmacogenetics 2004, 14:527–537.

27. Cavallari LH, Vaynshteyn D, Freeman KM, Wang D, Perera MA, Takahashi H, Drozda K, Patel SR, Jeong H: CYP2C9 promoter region single-nucleotide polymorphisms linked to the R150H polymorphism are functional suggesting their role in CYP2C9*8-mediated effects. Pharmacogenet Genomics 2013, 23:228–231.

28. Patel SR, Langaee TY, Wong SS, Cavallari LH: Pyrosequencing of the CYP2C9-1766T>C polymorphism as a means of detecting the CYP2C9*8 allele. Pharmacogenomics. 2014, pp. 1717–1722.

29. Liu Y, Jeong H, Takahashi H, Drozda K, Patel SR, Shapiro NL, Nutescu EA CL: Decreased warfarin clearance associated with the CYP2C9 R150H (*8) polymorphism. Clin Pharmacol Ther 2012, 91:660–665.

30. Allabi AC, Gala JL, Horsmans Y: CYP2C9, CYP2C19, ABCB1 (MDR1) genetic polymorphisms and phenytoin metabolism in a Black Beninese population. Pharmacogenet Genomics 2005, 15:779–786.

31. Liu Y, Jeong H, Takahashi H, Drozda K, Patel SR, Shapiro NL, Nutescu EA, Cavallari LH: Decreased warfarin clearance associated with the CYP2C9 R150H (*8) polymorphism. Clin Pharmacol Ther 2012, 91:660–665.

32. Tai G, Farin F, Rieder MJ, Dreisbach AW, Veenstra DL, Verlinde CLMJ, Rettie AE: In-vitro and in-vivo effects of the CYP2C9*11 polymorphism on warfarin metabolism and dose. Pharmacogenet Genomics 2005, 15:475–481.

33. Caudle KE, Rettie AE, Whirl-Carrillo M, Smith LH, Mintzer S, Lee MT, Klein TE CJCPIC: Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and HLA-B genotypes and phenytoin dosing. Clin Pharmacol Ther 2014, 96:542–548.

34. Swen JJ, Nijenhuis M, De Boer A, Grandia L, Maitland-Van Der Zee AH, Mulder H, Rongen GAPJM, Van Schaik RHN, Schalekamp T, Touw DJ, Van Der Weide J, Wilffert B, Deneer VHM, Guchelaar HJ: Pharmacogenetics: From bench to byte an update of guidelines. Clin Pharmacol Ther 2011, 89:662–673.

35. Scordo MG, Pengo V, Spina E, Dahl ML, Gusella M, Padrini R: Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. Clin Pharmacol Ther 2002,

72:702–710.

36. Dickmann LJ, Rettie AE, Kneller MB, Kim RB, Wood AJJ, Stein CM, Wilkinson GR, Schwarz UI: Identification and Functional Characterization of a New CYP2C9 Variant (CYP2C9*5) Expressed among African Americans. Mol Pharmacol 2001, 60:382–387.

37. Redman AR, Dickmann LJ, Kidd RS, Goldstein JA, Ritchie DM, Hon YY: CYP2C9 Genetic Polymorphisms and Warfarin. Clin Appl Thromb 2004, 10:149–154.

38. Gage BF, Bass AR, Lin H, Woller SC, Stevens SM, Al-Hammadi N, Li J, Rodríguez T, Miller JP, McMillin GA, Pendleton RC, Jaffer AK, King CR, Whipple BD, Porche-Sorbet R, Napoli L, Merritt K, Thompson AM, Hyun G, Anderson JL, Hollomon W, Barrack RL, Nunley RM, Moskowitz G, Dávila-Román V, Eby CS: Effect of Genotype-Guided Warfarin Dosing on Clinical Events and Anticoagulation Control Among Patients Undergoing Hip or Knee Arthroplasty: The GIFT Randomized Clinical Trial. JAMA 2017, 318:1115–1124.

39. Kimmel SE, French B GNCI: Genotype-guided dosing of vitamin K antagonists. N Engl J Med 2014, 370:1763–1764.

40. Drozda K, Wong S, Patel SR, Bress AP, Nutescu EA, Kittles RA, Cavallari LH: Poor warfarin dose prediction with pharmacogenetic algorithms that exclude genotypes important for African Americans. Pharmacogenet Genomics 2015, 25:73–81.

41. Hennessy S, Leonard CE, Freeman CP, Metlay JP, Xin Chu, Strom BL, Bilker WB: CYP2C9, CYP2C19, and ABCB1 genotype and hospitalization for phenytoin toxicity. J Clin Pharmacol 2009, 49:1483–1487.

42. DeLozier TC: Functional Characterization of Novel Allelic Variants of CYP2C9 Recently Discovered in Southeast Asians. J Pharmacol Exp Ther 2005, 315:1085–1090.

Table 1: Commercially Available *CYP2C9* Testing Platforms

CYP2C9 allele	Affymetrix PharmacoScan (RUO)*	Agena Biosciences iPLEX ADME (RUO)†	Autogenomics INFINITI‡ (CE-marked)	GenMark eSensor (FDA-cleared)§	BioFire Defense analyte-specific reagents)¶	Thermo Fisher OpenArray (V, RUO)*	TrimGen (FDA-cleared)‖
1B		x					
1C		x					
1D		x					
2	x	x	x	X	X		x
2C		x					
3	x	x	x	X	X		x
3A		x					
3B		x					
4	x	x	x				
5	x	x	x				
6	x	x	x				
7		x					
8	x	x					
9	x	x					
10	x	x					
11	x		x				
11A		x					
11B		x					
12	x	x					
13	x	x					
14		x					
15	x	x					
16	x	x					
17	x	x					
18	x	x					

19	x	x
20	x	x
21	x	x
22		x
23	x	x
24	x	x
25	x	x
26	x	x
27		x
28		x
29	x	x
30	x	x
31	x	x
32	x	x
33		x
34	x	x
36	x	
37	x	
38	x	
39	x	
40	x	
42	x	
43	x	
44	x	
45	x	
46	x	
47	x	
48	x	
49	x	
50	x	
51	x	
52	x	

53	x
54	x
55	x
56	x
57	x
58	x

Commercially available platforms as of 4/2/2019 and does not represent a comprehensive list. Inclusion herein does not represent an endorsement of any product or service by AMP.

*Thermo Fisher Scientific, (Waltham, MA).

†Agena Biocience (San Diego, CA).

‡AutoGenomics (Carlsbad, CA)

§GenMark Diagnostics (Carlsbad, CA).

¶BioFire Defense, LLC (Murray, UT).

‖TrimGen Genetic Diagnostics (Sparks, MD).

FDA, Food and Drug Administration; RUO, research use only; V, variable.

Table 2. Current Publicly Available Reference Materials for *CYP2C9.*

Allele	Coriell# (diplotype)
**2*	NA10854 (*2/*2)
	NA10831 (*1/*2)
**3*	NA10855 [*2/*3 (*18)]
	NA17290 [*1/*3 (*18)]
**4*	none
**5*	NA23275 (*5/*5)
	NA19908 (*1/*5)
	NA19178 (*5/*9)
**6*	NA19213 (*1/*6)
	NA19143 (*1/*6)
**7*	none
**8*	NA19226 (*1/*8)
	NA12815 (*1/*8)
**9*	NA07439 (*1/*9)
	NA19178 (*5/*9)
**10*	NA15245 (*10/*12)
**11*	NA19122 (*1/*11)
**12*	NA15245 (*10/*12)
**13*	none
**15*	none
**18*	NA19917 [*1/*1 (*18)]
	NA23405 [*1/*3 (*18)]

This is not a comprehensive list. Inclusion herein does not represent an endorsement of any product or service by AMP. For a complete list, see CDC website.

(https://wwwn.cdc.gov/clia/Resources/GETRM/default.aspx, last accessed 8/15/2018).

Table 3. *CYP2C9* Tier 1 variant alleles.

Allele	Allele Functional Status†	Defining Functional Variant	HGVS Nomenclature: NM_000771.3	HGVS Nomenclature: NG_008385.1‡	Reference Material Available	Multiethnic Allele Frequency
*2§	Decreased function	rs1799853	c.430C>T, p.Arg144Cys	g.8633C>T	Yes	0-12%
*3¶	Decreased function	rs1057910	c.1075A>C, p.Ile359Leu	g.47639A>C	Yes	1-11%
*5	Decreased function	rs28371686	c.1080C>G, p.Asp360Glu	g.47644C>G	Yes	0-1%
*6	No function	rs9332131	c.818del, p.Lys273Argfs*34	g.15625delA	Yes	0-1%
*8	Decreased function	rs7900194	c.449G>A, p.Arg150His	g.8652G>A	Yes	0-5%
*11	Decreased function	rs28371685	c.1003C>T, p.Arg335Trp	g.47567C>T	Yes	0-2%

† Citations for assignment of function can be found at https://www.pharmvar.org/gene/CYP2C9, last accessed 8/15/2018.

‡ CYP2C9 RefSeqGene.

§ Note that the defining variant of the *35 allele (c.374G>T, p.Arg125Leu) is likely in linkage disequilibrium with the defining *2 variant (c.430C>T, p.Arg144Cys)).

¶ Note that the defining *18 variant of the allele (c.1190A>C, p.Asp397Ala, rs72558193) is likely in linkage disequilibrium with the defining variant of *3 variant (c.1075A>C, p.Ile359Leu, rs1057910).

Table 4. *CYP2C9* Tier 2 variant alleles.

Allele	Allele Functional Status†	Defining Functional Variant	HGVS Nomenclature: NM_000771.3	HGVS Nomenclature: NG_008385.1‡	Reference Material Available	Multiethnic Allele Frequency
*12	Decreased function	rs9332239	c.1465C>T, p.Pro489Ser	g.55363C>T	Yes	0-0.3%
*13	Decreased function	rs72558187	c.269T>C, p.Leu90Pro	g.8301T>C	No	0-0.2%
*15	No function	rs72558190	c.485C>A, p.Ser162*	g.14125C>A	No	0-0.01%

† https://www.pharmvar.org/gene/CYP2C9, last accessed 8/15/2018.

‡ CYP2C9 RefSeqGene; forward relative to chromosome.